Exhibit E

6

OILS AND FATS

Capillary Column Gas Chromatographic Method for Analysis of Encapsulated Fish Oils and Fish Oil Ethyl Esters: Collaborative Study

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A gas chromatographic (GC) method using a capillary column for analysis of encapsulated fish oils and ethyl esters was studied collaboratively in 21 laboratories. Each collaborator analyzed 6 soft-gelatin encapsulated samples; 5 were triacylglycerol oils (one was a blind duplicate), and one was an ethyl ester concentrate of omega-3 (n-3) polyunsaturates. Constituent fatty acids of the oils were converted to methyl esters by base-catalyzed transesterification of the oils; any free acids in the oils were esterified by subsequent reaction with BF_3/CH_3OH. The ethyl ester concentrate required no further derivatization. Results were reported as area percentages of 24 analytes of nutritional or biochemical interest. In addition, weights (mg/g sample) of EPA (all-*cis*-5,8,11,14,17-eicosapentaenoic acid or 20:5n-3) and DHA (all-*cis*-4,7,10,13,16,19-docosahexaenoic acid or 22:6n-3) were determined through the use of the internal standards, respectively, methyl tricosanoate (23:0) and ethyl 23:0, for the methyl and ethyl esters. The only instrumentation specifically required was a flexible fused silica capillary GC column coated with a bonded polyglycol such as Carbowax-20M, an oxygen scrubber installed in the carrier gas supply line, and a flame ionization detector (FID). Most of the collaborators experienced little difficulty in applying the method, and, of 2526 values reported, only 4.3% were identified as outlier values. The reproducibility relative standard deviations (RSD_R) compared favorably in most instances with, or were substantially better than, those of 2 earlier collaborative studies of fish oils. Because the variances were homogeneous, standard deviations and relative standard deviations determined on the area percent analyses of the blind duplicate oils were pooled to give the following mean values: s_r = 0.15, RSD_r = 4.88%, s_R = 0.41, and RSD_R = 12.91%. Analytes that rarely occur at greater than 0.5% in marine oils (22:0, 22:4n-6, 22:5n-6, 24:0, and 24:1) were not included in these calculations. The method was adopted first action by AOAC International as an American Oil Chemists' Society (AOCS)-AOAC method.

Current consumer interest in fish oils and related preparations sold as over-the-counter nutritional supplements (1) creates a need for accurate labeling of the products for the active ingredients, ostensibly EPA (all-*cis*-5,8,11,14,17-eicosapentaenoic acid or 20:5n-3) and DHA (all-*cis*-4,7,10,13,16,19-docosahexaenoic acid or 22:6n-3). The information needed for this labeling requires the application of the most appropriate fatty acid technology, given the complex chemical composition of fish oils and their derivatives.

Packed column gas chromatography (GC) is suitable for the analysis of most vegetable oils, but these columns lack the resolution necessary to separate the 60–80 fatty acids commonly present in fish oils. Therefore, capillary column GC, which has been used in marine lipid research for 2 decades, was deemed more suitable for quality control of commercial products. Improved separation of all components by capillary column GC

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The recommendation was approved by the General Referee and the Committee on Foods I and was adopted by the Official Methods Board of AOAC. See "Changes in Official Methods of Analysis" (1992) *J. AOAC Int.* 75, 223–225.
Mention of trade names, commercial firms, or specific products or instrumentation is for identification purposes only and does not constitute endorsement by the National Marine Fisheries Service.
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simultaneously improves quantitation in area percentages of EPA and DHA by the GC electronic integrator.

Although the results of GC analyses of fatty acid methyl esters are usually reported as area percentages of eluted components, the area percentages of EPA and DHA must be converted to absolute weights per gram of sample for informative labeling of nutritional supplements. This conversion requires the use of internal or external standards, and for accuracy, correction factors for the flame ionization detector (FID) response must be applied.

The purpose of the present international collaborative study was 2-fold. The first was to assess the effectiveness of polyglycol-coated capillary columns in separating fish oil fatty acids (as methyl and ethyl esters) of major nutritional or biochemical importance. The second purpose was to test the suitability of methyl and ethyl 23:0 as internal standards in the calculation of the absolute weights of EPA and DHA in fish oils and ethyl esters derived from that source.

Collaborative Study

Twenty-one laboratories participated in the study. Each collaborator received 3 capsules, each of 5 fish oils (including a blind duplicate of 1 oil) and 1 ethyl ester concentrate of n-3 polyunsaturates. Methyl and ethyl 23:0 were supplied as internal standards. The participants were instructed to use 2 capsules of each sample for analyst familiarization and instrument optimization and to submit results of a single analysis of the third capsule of each sample. Included with instructions, the study protocol, and the required calculations were 2 data report forms. The first provided space for a description of instrumentation, column dimensions and history, and operating parameters. The second was for listing the area percentages of 24 analytes of particular interest and the calculated weights (mg/g sample) of EPA and DHA in the 6 samples. A chromatogram of methyl esters of commercially encapsulated cod liver oil was included as an aid to peak identification and as an indicator of the resolution that could be expected from a properly operated polyglycol capillary column. The resolution of methyl docosapentanoate (22:5n-3) and methyl DHA should be at least 4.

991.39 Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters—Gas Chromatographic Method

First Action 1991

AOCS–AOAC Method

Method Performance:
See Table 991.39 for method performance data.

A. Principle

Samples are weighed into Teflon-lined screw-cap glass tubes that contain appropriate internal standards. Fatty acids of oil samples are derivatized to methyl esters; ethyl ester samples require no derivatization. Prepared samples are analyzed by GC instrument equipped with fused silica column coated with bonded polyglycol liquid phase, oxygen scrubber in carrier gas line, and flame ionization detector. Method determines area percentages of 24 fatty acids and absolute weights (mg/g sample) of EPA (all-*cis*-5,8,11,14,17-eicosapentaenoic acid or 20:5n-3) and DHA (all-*cis*-4,7,10,13,16,19-docosahexaenoic acid or 22:6n-3).

B. Apparatus

(a) *Gas chromatograph.*—With flame ionization detector, capillary column injection system (split mode preferred at split ratio of 1:50), and suitable data processor. (*Note*: In fish oil analyses, samples are usually sufficient to permit operation in split mode.) Operating conditions: temperatures—injection port 250°; detector 270°; oven programmed from 170 to 225° at 1°/min (no initial or final hold). Helium or hydrogen carrier gas (99.99% pure, or better) with oxygen scrubber in line.

(b) *GC column.*—Fused silica, 30 m × 0.25 mm (or 0.32 mm) coated with bonded polyglycol, based on Carbowax-20M (e.g., SUPELCOWAX-10, or equivalent column that provides same elution pattern as that illustrated in Fig. 991.39 and baseline separation of 21:5n-3, 23:0, and 22:4n-6).

(c) *Constant temperature water bath.*—Maintained at 100°. Dry heater block may be used.

(d) *Glass tubes.*—16 × 125 mm. With leak-tight, Teflon-lined screw caps.

(e) *Vials.*—2 mL, with screw cap or crimp cap (for autosampler).

(f) *Analytical balance.*—Accurate to ±0.0001 g.

(g) *Dry nitrogen source.*

(h) *Glassware.*—Volumetric flasks, 25 and 100 mL; volumetric pipets, 1 and 2 mL; Pasteur pipets.

C. Reagents

(a) *Boron trifluoride.*—BF_3, 12% in methanol. Two mL amber glass ampoules (Supelco, Inc., Cat. No. 3-3020, or equivalent reagent, sealed in amber glass ampoules for extended shelf life). (*Caution*: BF_3 in methanol is a corrosive reagent and must be handled with care. Avoid eye and skin contact by use of protective shield and rubber gloves. Use only in properly operating fume hood.)

(b) *23:0 Methyl and ethyl esters.*—Reagents of 99+% purity as determined by TLC and GC analyses [Nu Chek Prep, Inc., Elysian, MN, Cat. No. N-23-M (methyl ester) and Cat. No. N-23-E (ethyl ester), or equivalent]. [Note: On request, the Charleston Laboratory, Southeast Fisheries Center, National Marine Fisheries Service, PO Box 12607, Charleston, SC 29422-0607, will provide capsules of collaborative study Sample 1 (steam-deodorized menhaden oil) for use in optimizing GC equipment.]

(c) *Reagent grade chemicals.*—Sodium hydroxide, methanol, isooctane, sodium chloride. (*Caution*: See safety notes on sodium hydroxide, methanol, and isooctane in Appendix, *Official Methods of Analysis* (1990) 15th Ed., AOAC, Arlington, VA.)

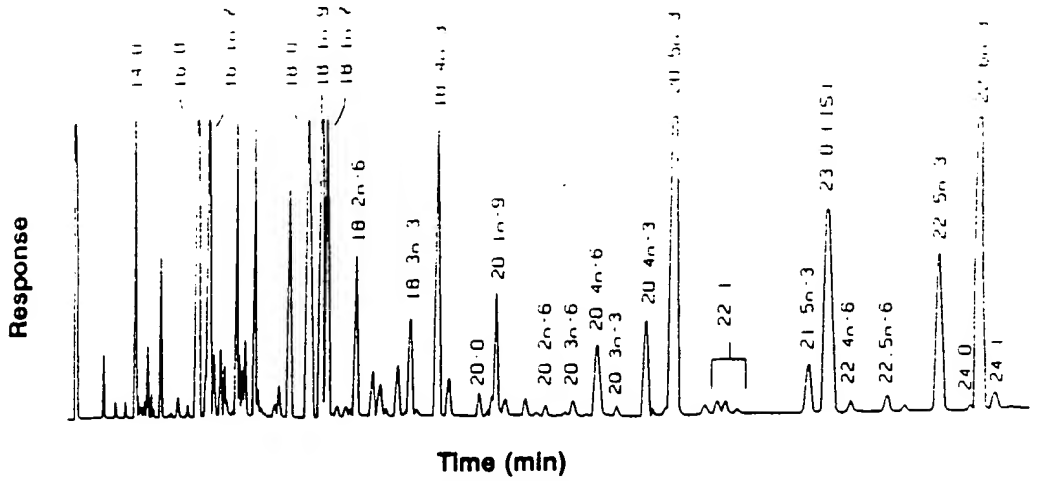

Figure 991.39. Temperature-programmed GC separation of menhaden oil fatty acid methyl esters on flexible fused silica column coated with bonded Carbowax 20M.

D. Preparation of Solutions

(a) *Alcoholic sodium hydroxide.*—0.5N. Dissolve 2.0 g NaOH in methanol and dilute to 100 mL with methanol.

(b) *Sodium chloride.*—Saturated solution. Dissolve 36 g NaCl in 100 mL H_2O.

E. Preparation of Standards

Accurately weigh ca 25 mg (± 0.1 mg) of 23:0 methyl or ethyl ester internal standard (IS) into 25 mL volumetric flask and dilute to volume with isooctane. Pipet 1.0 mL portions into screw-cap glass tubes and evaporate solvent in gentle stream of nitrogen. Store tubes in freezer if not to be used immediately.

F. Sample Preparation and Analysis

(a) *Oils.*—(*Note*: "Oil" applies to all encapsulated materials, including nonesterified fatty acids, with exception of ethyl esters.)

Accurately weigh ca 25 mg (±0.1 mg) oil into glass tube containing methyl ester IS, E. Add 1.5 mL 0.5N methanolic NaOH, blanket with nitrogen, cap, mix, and heat 5 min at 100°. Cool, add 2 mL BF_3 in methanol, C(a), blanket with nitrogen, cap tightly, mix, and heat 30 min at 100°. Cool mixture to 30–40°, add 1 mL isooctane, blanket with nitrogen, cap, and shake vigorously for 30 s while still warm.

Immediately add 5 mL saturated NaCl solution, blanket with nitrogen, cap, and agitate thoroughly. Cool to room temperature. When isooctane layer separates from aqueous lower phase, transfer isooctane layer to a clean glass tube, blanket with nitrogen, and cap.

Extract aqueous lower phase a second time with an additional 1 mL isooctane. Combine isooctane extracts and concentrate to ca 1 mL in stream of dry nitrogen.

Inject 1–2 µL into GC system.

(b) *Ethyl esters.*—Accurately weigh ≤15 mg (±0.1 mg) ethyl ester (usually more concentrated) into glass tube containing appropriate ester IS, E. Add 1 mL isooctane, blanket with nitrogen, cap, and mix thoroughly.

Inject 1–2 µL into GC system. If peak height of IS is ≤0.5 that of EPA or DHA peak, repeat analysis, using 2.0 mL IS.

G. Calculations

(a) *Area percentage.*—Calculate area percentages of fatty acid methyl esters or ethyl esters as follows:

$$\text{Area \% fatty acid}_X = [A_X/(A_T - A_{IS})] \times 100$$

where A_X = area counts of methyl or ethyl ester X; A_T = total area counts for chromatogram; and A_{IS} = area counts of IS.

(b) *Weight of EPA and DHA in oils.*—Calculate EPA or DHA, mg/g oil, as follows:

$$\text{EPA or DHA, mg/g} = [(A_X \times W_{IS} \times CF_X)/(A_{IS} \times W_S \times 1.04)] \times 1000$$

where A_X = area counts of EPA or DHA; A_{IS} = area counts of internal standard; CF_X = theoretical detector correction factor for EPA or DHA (0.99 for EPA, 0.97 for DHA); W_{IS} = weight of IS added to sample, mg; W_S = sample weight, mg; and 1.04 is factor necessary to express result as mg fatty acid/g oil (rather than as methyl ester).

(c) *Weight of EPA and DHA in ethyl esters.*—Calculate EPA or DHA, mg/g esters, as follows:

$$\text{EPA or DHA, mg/g} = [(A_X \times W_{IS} \times CF_X)/(A_{IS} \times W_S \times 1.08)] \times 1000$$

where terms are same as in (b), except use 1.08, factor necessary to express result as mg fatty acid/g ethyl ester (rather than as ethyl ester).

Ref.: AOCS Official Method Ce 1b-89. JAOAC 75, May/June issue (1992)

Table 991.39. Method performance for 991.39, fatty acids in encapsulated fish oils and ethyl esters[a]

Fatty acid	s_R	RSD_R, %	s_r	RSD_r, %	Fatty acid	s_R	RSD_R, %
	Fish oils, area %					Ethyl ester concentrate, area %	
14:0	0.58–0.98	8.31–13.12	0.49	5.8	14:0	0.06	17.07
16:0	0.44–1.91	5.66–10.02	0.54	2.9	16:0	0.11	12.24
16:1	0.55–2.59	6.80–26.73	0.51	4.3	16:1	0.10	30.96
18:0	0.05–0.42	3.05–14.43	0.06	1.8	18:0	0.15	7.00
18:1	0.23–0.68	1.92–6.71	0.19	1.6	18:1	0.50	5.72
18:2n-6	0.05–0.13	2.37–10.69	0.01	1.2	18:2n-6	0.06	7.93
18:3n-3	0.04–0.17	3.86–23.14	0.04	5.6	18:3n-3	0.08	12.85
18:4n-3	0.07–0.24	2.43–6.30	0.04	1.5	18:4n-3	0.15	7.62
20:0	0.02–0.17	7.78–84.25	0.01	5.5	20:0	0.03	10.12
20:1	0.15–0.45	4.46–18.13	0.13	7.3	20:1	0.45	3.19
20:2n-6	0.01–0.08	10.29–25.77	0.01	8.6	20:2n-6	0.10	32.42
20:3n-6	0.03–0.07	16.08–67.73	0.03	17.4	20:3n-6	0.11	35.22
20:3n-3	0.04–0.07	27.47–47.93	0.01	3.1	20:3n-3	0.07	29.32
20:4n-6	0.07–0.19	8.86–43.83	0.02	2.5	20:4n-6	0.09	7.37
20:4n-3	0.06–0.15	4.91–13.77	0.04	3.5	20:4n-3	0.15	7.86
20:5n-3	0.43–2.06	5.48–9.78	0.25	1.9	20:5n-3	1.54	5.83
22:0	0.02–0.38	11.77–141.42	0.02	8.9	22:0	0.07	73.94
22:1	0.14–0.80	8.88–17.24	0.11	7.9	22:1	0.50	4.68
22:4n-6	0.08–0.17	38.29–93.55	0.06	26.3	22:4n-6	0.41	84.99
22:5n-6	0.04–0.08	13.14–50.60	0.03	18.6	22:5n-6	0.14	43.08
22:5n-3	0.10–0.32	8.84–16.20	0.13	6.7	22:5n-3	0.36	8.52
22:6n-3	0.69–1.44	7.50–16.09	0.29	3.7	22:6n-3	1.40	7.51
24:0	0.02–0.14	48.38–100.00	0.01	10.9	24:0	0.11	158.70
24:1	0.07–0.73	41.22–102.20	0.03	7.4	24:1	0.23	35.16
	Fish oils, absolute weight (mg/g sample)					Ethyl ester concentrate, absolute weight (mg/g sample)	
20:5n-3	2.98–31.10	5.38–19.75	7.17	5.9	20:5n-3	20.40	9.15
22:6n-3	2.60–13.27	4.24–12.60	3.68	5.3	22:6n-3	14.15	8.97

[a] s_R and RSD_R for fish oils are ranges of values obtained in the collaborative study of 4 different fish oils. RSD_R values are elevated for analytes that rarely exceed 0.1–0.2% of total analytes (20:0, 20:3n-6, 22:0, 22:4n-6, 22:5n-6, 24:0, and 24:1).

Results

Details of equipment and some of the operating parameters used by the collaborators are listed in Table 1. All of the collaborators used helium carrier gas, with one exception; Collaborator 18 used hydrogen. Most of the columns used had been in operation for 1–10 months before the study began, but a number of collaborators reported the use of new columns, and one, Collaborator 10, reported that the column had been in service for 5 years. All but Collaborators 10, 15, 16, and 17 operated in split mode; split ratios varied from 1:50 to 1:100.

No restrictions were placed on the selection of GC instrumentation by the study participants beyond the mandatory use of a flexible fused silica capillary column coated with a bonded polyglycol liquid phase, an oxygen scrubber in the carrier gas line to protect the column, and a flame ionization detector. However, Collaborators 17 and 21 used columns coated with DB-225, which is not a polyglycol but is, rather, a liquid phase composed of 50% cyanopropylphenyl and 50% methyl silicone. Consequently, these collaborators were considered to be "procedural deviates" (2), and their data were not used in the statistical calculations.

The raw data submitted by the collaborators for the 6 samples and the statistical calculations of reproducibility between laboratories are listed in Tables 2–7. Collaborator 14 submitted a typewritten table of results for the 6 samples. Reported area percentages for Samples 1 and 2 were virtually identical, although Sample 1 was steam-deodorized menhaden oil and Sample 2 was the ethyl ester n-3 concentrate. However, the calculated weight data reported for EPA and DHA in Sample 2 were almost twice as great as those reported for Sample 1. This indicates that Collaborator 14 did, in fact, properly analyze Sample 2 but inadvertently submitted erroneous area percent data for this sample. Despite 2 requests, no correct report on Sample 2 was received from this collaborator.

Collaborator 18 initially reported the weights of EPA and DHA as ranging from 0.06 to 0.216 mg/g in the 6 samples. When contacted and asked to verify these data, the collab-

Table 1. Instrumentation and operating parameters used by collaborative study participants

Laboratory	GC	Column		Temperatures,°C			Program
		Liquid phase	Dimensions	Inj. port	Detector	Column	
1	HP-5880[a]	Carbowax-20M	25 m × 0.20 mm	250	270	170–225	1°/min
2	HP-5880	Carbowax-20M	50 m × 0.25 mm	275	300	193–225	hold 35 min then 1°/min to 225°
3	HP	DB-Wax	30 m × 0.25 mm	225	270	140–240	hold 2 min then 4°/min to 240°
4	PE-8420[b]	SUPELCOWAX-10	30 m × 0.32 mm	250	270	190–240	hold 8 min then 3°/min to 240°
5	HP-5880	SUPELCOWAX-10	30 m × 0.32 mm	250	270	170–225	1°/min
6	HP-5890	SUPELCOWAX-10	30 m × 0.32 mm	250	270	170–225	1°/min
7	HP-5840	SUPELCOWAX-10	30 m × 0.25 mm	250	270	170–225	1°min
8	HP-5790	DB-Wax	30 m × 0.32 mm	200	250	100–230	hold 2 min then 20°/min to 180° then 2°/min to 230°.
9	HP-5880	SUPELCOWAX-10	30 m × 0.32 mm	250	270	170–225	1.5°/min
10	HP	SUPELCOWAX-10	30 m × 0.32 mm	250	270	170–225	1°/min
11	HP	DB-Wax	30 m × 0.32 mm	240	260	170–250	hold 2 min (no rate given)
12	HP-5880	SUPELCOWAX-10	30 m × 0.32 mm	250	270	170–225	1°/min
13	Carlo Erba	SUPELCOWAX-10	30 m × 0.32 mm	250	270	170–245	hold 30 s then 5°/min to 245°
14	Carlo Erba V6000	CP-Wax 58	50 m × 0.25 mm	250	270	190–210	hold 15 min then 1°/min to 210°
15	PE	SUPELCOWAX-10	60 m × 0.32 mm	250	270	180–230	1.5°/min
16	HP	SUPELCOWAX-10	30 m × 0.32 mm	250	270	170–225	1°/min
17	PE-8420	DB-225	30 m × 0.32 mm	30	270	30–200	30°/min to 120°, hold 5 min, then 5°/min to 200°
18	Varian	SUPELCOWAX-10	30 m × 0.32 mm	250	270	170–225	1°/min
19	HP-5890	DB-Wax	30 m × 0.32 mm	250	270	170–225	1°/min
20	PE-8500	SUPELCOWAX-10	30 m × 0.32 mm	250	250	185–205	hold 15 min then 2°/min to 205°
21	Shimadzu	DB-225	30 m × 0.25 mm	275	275	180–236	2°/min to 236°, hold 9 min

[a] Hewlett-Packard.
[b] Perkin-Elmer.

Table 2. Collaborative study results of analysis of Sample 1 (steam-deodorized menhaden oil)

	Composition, area %																									
	Laboratory																									
Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17[a]	18	19	20	21[a]	Mean	s_R[b]	RSD_R, %[c]	R[d]	Outliers/ No. labs.
14:0	7.6	8.2	8.0	9.0	6.3	8.3	7.4	8.0	8.6	8.4	7.3	8.3	8.7	8.0	10.1	8.1	5.3	10.7	9.2	8.5	7.8	8.34	0.98	11.70	2.7	0/19
16:0	18.0	18.9	19.0	20.0	15.8	18.8	18.0	18.5	20.0	19.4	15.4	18.6	19.4	18.7	20.5	19.0	8.6	24.4[e]	19.7	18.7	18.8	18.69	1.31	6.98	3.7	1/19
16:1	11.4	11.5	11.6	12.3	9.7	11.4	11.2	12.4	12.2	11.8	10.3	10.9	11.9	11.8	12.8	15.9	16.9	16.6	12.2	11.4	10.8	12.06	1.65	13.64	4.6	0/19
18:0	3.1	3.2	3.2	2.9	3.1	3.0	3.1	3.0	3.1	3.1	3.4	3.1	3.1	3.1	2.5[e]	3.2	2.4	3.7[e]	3.0	3.0	3.1	3.10	0.11	3.60	0.3	2/19
18:1	12.0	12.2	12.2	11.6	12.0	11.6	11.9	11.8	12.3	11.9	12.1	11.9	12.0	11.9	10.5[e]	12.2	10.2	14.4[e]	11.7	11.5	11.8	11.92	0.23	1.92	0.6	2/19
18:2n-6	1.2	1.2	1.2	1.1	1.2	1.2	1.2	1.2	1.2	1.2	1.3	1.2	1.2	1.2	1.0	1.2	3.3	1.5[e]	1.2	1.1	1.2	1.18	0.07	5.57	0.2	1/19
18:3n-3	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.3[e]	0.8	0.5[e]	0.8	0.7	0.8	1.6	1.1[e]	0.8	0.8	0.8	0.79	0.04	4.88	0.1	3/19
18:4n-3	2.9	3.0	3.0	2.7	3.0	2.9	2.9	2.9	2.9	2.9	3.3	2.9	2.9	2.9	2.5[e]	3.6[e]	3.3	3.6[e]	2.9	2.8	2.8	2.90	0.07	2.43	0.2	3/19
20:0	0.2	—[f]	0.2	0.2	—	—	0.2	0.2	—	0.2	0.9	0.2	0.2	0.2	—	0.2	1.5[e]	0.5[e]	0.2	—	0.2	0.21	0.02	7.78	0.0	2/13
20:1	2.0	1.6	2.0	1.7	2.4[e]	1.7	1.9	1.9	1.7	1.8	2.1	1.9	1.9	1.8	1.2[e]	1.8	1.0	1.6	1.7	1.8	1.9	1.81	0.15	8.04	0.4	2/19
20:2n-6	0.2	—	0.1	0.1	0.2	0.2	0.2	0.2	—	0.2	—	0.2	0.1	0.1	—	—	0.3	—	0.1	0.3	0.2	0.14	0.04	27.71	0.1	0/14
20:3n-6	0.2	—	0.1	0.2	0.3	0.2	0.2	0.2	—	0.2	—	0.2	0.2	0.2	0.2	0.2	0.4	—	0.1	0.3	0.2	0.18	0.06	40.63	0.2	0/16
20:3n-3	—	—	0.1	0.2	0.3	0.2	0.2	0.2	—	0.1	0.2	—	0.1	0.1	0.1	0.9[e]	0.9	—	0.1	0.1	0.1	0.15	0.05	41.73	0.1	1/14
20:4n-6	0.8	0.9	0.9	0.7	1.0	0.9	0.9	0.8	0.8	0.8	1.0	0.9	0.9	0.8	0.6	—	0.2[e]	0.9	0.8	0.8	0.8	0.84	0.09	10.52	0.2	1/19
20:4n-3	1.3	1.3	1.3	1.2	1.5	1.3	1.3	1.3	1.2	1.3	1.6[e]	1.3	1.2	1.3	0.8[e]	1.3	0.4	1.3	1.2	1.2	1.4	1.29	0.06	4.91	0.2	2/19
20:5n-3	14.7	14.6	14.0	13.2	15.5	13.4	14.3	13.0	14.7	12.6	13.3	14.0	13.7	13.7	10.0[e]	14.5	12.8	13.5	12.9	12.8	14.1	13.79	0.79	5.76	2.2	1/19
22:0	0.5	—	0.2	0.3	—	—	0.2	0.2	—	0.1	0.1	—	0.1	0.2	—	—	0.6	—	0.2	—	0.1	0.19	0.13	69.73	0.4	0/12
22:1	1.0	1.4	1.5	1.5	1.8	1.3	1.4	1.3	1.2	1.5	1.9	1.4	1.3	1.6	1.3	0.2[e]	0.7	—	1.2	1.9	0.9	1.43	0.25	17.24	0.7	1/18
22:4n-6	—	—	0.1	0.5	0.3	0.3	0.2	0.2	—	0.2	0.1	0.1	0.1	0.1	—	0.7	1.2	—	0.1	0.3	0.1	0.23	0.17	75.55	0.5	0/14
22:5n-6	0.2	—	0.2	0.3	0.3	0.2	0.2	0.2	—	0.1	0.2	—	0.1	0.1	—	—	0.6	—	0.1	0.3	—	0.17	0.08	45.59	0.2	0/14
22:5n-3	2.4	2.3	2.0	1.9	2.4	2.0	2.2	1.9	2.1	1.8	2.5	2.2	1.9	1.9	0.7	2.2	0.3[e]	1.2	1.8	2.1	2.2	2.05	0.29	14.59	0.8	1/19
22:6n-3	9.3	9.0	8.1	7.9	9.7	7.7	8.6	7.5	8.7	7.2	8.9	8.5	8.0	7.7	4.9	8.5	8.9	4.9	6.9	7.6	8.8	7.87	1.27	16.09	3.5	0/19
24:0	—	—	—	—	—	—	0.1	0.1	0.2	0.1	0.1	—	—	0.1	—	0.6	0.8	—	—	—	0.3	0.10	0.07	69.96	0.2	0/09
24:1	0.3	—	0.1	—	0.5	0.2	0.4	0.4	0.4	0.2	0.5	0.6	0.1	0.3	0.4	0.3	0.7	—	0.3	0.4	0.3	0.33	0.14	42.42	0.4	0/16
	mg/g sample																									
20:5n-3	105.0	101.2	119.5	119.1	111.7	117.0	111.6	118.1	114.0	119.7	118.0	189.4[e]	109.5	116.0	130.1	114.0	97.9	—	125.0	110.8	—	115.35	6.95	6.04	19.4	1/18
22:6n-3	65.0	61.2	67.9	71.1	67.4	66.4	66.3	68.0	66.0	67.4	71.0	112.0[e]	62.6	63.3	61.8	66.0	66.7	—	65.7	64.7	—	65.99	2.80	4.24	7.8	1/18

[a] Polyglycol column not used. Data are not included in statistical calculations.
[b] Reproducibility standard deviation.
[c] Reproducibility relative standard deviation.
[d] Reproducibility value ($2.8 \times s_R$).
[e] Outlier value, determined by Dixon and/or Grubbs tests, not used in statistical calculations.
[f] Denotes a value reported as "—," "< 0.05," "0," or "tr."

Table 3. Collaborative study results of analysis of Sample 2 (SuperEPA 500®, ethyl ester)

	Composition, area %																									
	Laboratory																									
Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14[a]	15	16	17[a]	18	19	20	21[a]	Mean	s_R[b]	RSD_R, %[c]	R[d]	Outliers/ No. labs.
14:0	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.4	—	0.5	0.4	0.3	0.8[e]	0.5	0.5	0.4	0.38	0.06	17.07	0.2	1/18
16:0	0.9	0.9	0.9	0.8	0.7	0.8	0.8	0.9	0.9	1.0	1.1	0.9	0.9	—	1.1	1.0	0.6	1.9[e]	1.0	1.1	0.8	0.92	0.11	12 24	0.3	1/18
16:1	0.4	0.2	0.2	0.3	0.2	—	0.2	0.3	0.3	0.3	0.3	—	0.2	—	0.5	0.4	1.1[e]	0.8	0.3	0.5	0.2	0.31	0.10	30.96	0.3	1/16
18:0	2.0	2.2	2.2	2.0	2.0	2.1	2.0	2.1	2.0	2.3	2.5	2.2	2.2	—	2.0	2.2	1.8	3.0[e]	2.4	2.0	2.0	2.14	0.15	7.00	0.4	1/18
18:1	8.8	8.7	8.7	8.8	7.9	8.2	8.0	8.8	8.3	9.3	9.8	8.4	9.1	—	8.7	8.6	8.1	13.3[e]	9.4	8.5	8.3	8.69	0.50	5.72	1.4	1/18
18:2n-6	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.8	0.9	0.7	0.8	—	0.8	0.8	0.9	1.2[e]	0.8	0.8	0.8	0.76	0.06	7.93	0.2	1/18
18:3n-3	0.6	0.7	0.5	0.6	0.6	0.6	0.7	0.6	0.6	0.7	0.4	0.2[e]	0.6	—	0.7	0.6	1.0	1.0[e]	0.6	0.6	0.6	0.61	0.08	12.85	0.2	2/18
18:4n-3	1.9	1.9	1.9	1.8	1.7	1.8	1.7	1.8	2.0	2.0	2.2	1.9	1.9	—	1.9	2.2	1.4	3.0[e]	2.1	2.0	1.8	1.93	0.15	7.62	0.4	1/18
20:0	0.3	—[f]	0.3	0.3	—	—	0.3	0.3	0.3	0.3	0.8[e]	—	0.3	—	—	0.3	1.4[e]	0.4	0.3	0.5	0.3	0.32	0.03	10.12	0.1	2/13
20:1	14.1	14.3	14.1	13.9	13.7	14.0	13.9	13.7	14.1	14.0	15.1	14.5	14.6	—	11.9[e]	15.0	9.4	14.1	14.4	13.3	13.4	14.04	0.45	3.19	1.3	1/18
20:2n-6	0.1	—	0.4	0.3	0.4	—	0.4	0.4	0.3	0.4	0.1	—	0.4	—	0.3	—	3.2[e]	—	0.3	0.8	0.3	0.30	0.10	32.42	0.3	1/13
20:3n-6	0.2	—	0.4	0.3	0.6	—	0.2	0.3	0.2	0.4	0.4	0.3	0.3	—	—	0.3	1.7[e]	0.3	—	0.9	0.2	0.32	0.11	35.22	0.3	1/14
20:3n-3	0.2	—	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	—	0.2	—	0.3	1.3[e]	—	0.4	0.1	0.6[e]	0.3	0.23	0.07	29.32	0.2	2/16
20:4n-6	1.2	1.3	1.2	1.3	1.3	1.2	1.3	1.2	1.2	1.3	1.9	1.2	1.2	—	1.2	—	2.1	1.5	1.2	1.7[e]	1.2	1.25	0.09	7.37	0.3	1/17
20:4n-3	1.8	1.9	2.0	1.9	1.9	1.7	1.8	1.8	1.8	1.8	2.3	1.8	1.9	—	1.7	1.7	—	2.0	2.0	2.7[e]	1.7	1.86	0.15	7.86	0.4	1/18
20:5n-3	26.7	27.4	26.5	27.6	25.5	26.3	26.0	25.2	27.1	26.4	22.6	27.2	25.9	—	24.4	26.6	27.5	29.9	27.7	25.3	27.8	26.36	1.54	5.83	4.3	0/18
22:0	—	—	0.1	0.2	—	—	—	0.2	—	0.1	0.1	—	—	—	—	—	0.9	—	—	—	—	0.10	0.07	73.94	0.2	0/07
22:1	10.8	11.4	10.7	10.8	10.4	10.6	10.8	10.2	11.1	9.9	11.0	11.2	10.7	—	9.4	10.5	10.5	7.8[e]	10.0	10.7	10.3	10.59	0.50	4.68	1.4	1/18
22:4n-6	—	—	0.3	0.4	0.5	—	0.2	0.2	1.3	0.3	0.3	—	0.3	—	—	1.3	1.5	—	—	0.4	—	0.48	0.41	84.99	1.1	0/11
22:5n-6	0.3	—	0.3	0.3	0.4	—	0.3	0.3	0.3	0.2	0.3	—	0.3	—	0.7	0.6	0.5	—	0.2	0.3	—	0.33	0.14	43.08	0.4	0/14
22:5n-3	4.4	4.7	4.5	4.3	4.7	4.2	4.3	4.3	4.2	4.2	4.6	4.6	4.5	—	3.7	4.2	—	3.3	4.3	3.8	4.1	4.27	0.36	8.52	1.0	0/18
22:6n-3	20.4	19.8	19.0	19.6	18.5	19.0	19.7	18.3	20.6	17.9	17.5	20.1	18.6	—	16.2	19.6	22.9	15.3	18.3	17.9	20.5	18.68	1.40	7.51	3.9	0/18
24:0	—	—	—	—	—	—	—	0.1	—	—	—	0.3	—	—	—	—	—	—	—	—	—	0.02	0.11	158.70	0.3	1/02
24:1	0.6	—	0.7	—	1.1	0.6	0.7	0.7	0.8	—	0.2	0.7	0.3	—	—	0.8	0.5	—	0.6	—	0.6	0.64	0.23	35.16	0.6	0/12
	mg/g sample																									
20:5n-3	207.0	245.6	218.7	223.8	258.6	211.0	222.9	234.1	225.0	243.4	178.0	220.2	339.1[e]	—	190.3	220.0	262.1	—	239.0	231.1	—	223.11	20.40	9.15	57.1	1/17
22:6n-3	155.0	176.4	153.5	157.0	181.1	149.0	162.6	170.3	168.0	161.8	135.0	159.4	225.8[e]	—	123.5	159.0	213.9	—	155.0	159.8	—	157.90	14.15	8.97	39.6	1/17

[a] Polyglycol column not used. Data are not included in statistical calculations.
[b] Reproducibility standard deviation.
[c] Reproducibility relative standard deviation.
[d] Reproducibility value ($2.8 \times s_R$).
[e] Outlier value, determined by Dixon and/or Grubbs tests, not used in statistical calculations.
[f] Denotes a value reported as "—," "< 0.05," "0," or "tr."

Table 4. Collaborative study results of analysis of Sample 3 (cod liver oil)

Fatty acids	Composition, area % — Laboratory 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17[a]	18	19	20	21[a]	Mean	s_R[b]	RSD_R, %[c]	R[d]	Outliers/ No. labs.
14:0	5.2	5.4	5.6	6.5	4.4	5.2	4.6	5.4	5.2	5.8	5.7	5.8	6.0	5.8	7.5	5.5	4.0	6.7	6.2	5.7	5.3	5.71	0.75	13.12	2.1	0/19
16:0	12.7	12.9	13.5	14.7	10.9	12.9	12.1	13.0	13.2	13.7	12.2	13.8	14.2	13.6	15.3	13.3	9.9	17.8[e]	14.1	12.9	13.3	13.27	1.00	7.51	2.8	1/19
16:1	8.6	7.5	8.6	9.7	7.2	8.4	7.9	9.5	8.6	8.8	8.6	8.8	9.2	9.2	10.2	10.3	11.1	12.5[e]	9.3	8.4	7.8	8.82	0.83	9.39	2.3	1/19
18:0	2.3	2.3	2.4	2.3	2.3	2.3	2.3	2.3	2.3	2.4	2.4	2.4	2.3	2.3	2.1	2.4	1.9	3.3[e]	2.3	2.2	2.3	2.30	0.07	3.05	0.2	1/19
18:1	18.3	17.7	18.4	18.9	17.3	17.8	17.7	18.1	18.4	18.1	17.5	18.4	18.5	18.3	17.7	18.9	12.5	24.3[e]	17.6	17.1	18.1	18.03	0.51	2.82	1.4	1/19
18:2n-6	2.0	2.0	2.1	2.0	2.0	2.0	2.0	2.0	2.0	2.1	2.1	2.1	2.1	2.1	2.0	2.1	8.2[e]	2.6	2.1	2.0	2.0	2.04	0.05	2.37	0.1	1/19
18:3n-3	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0	0.9	1.0	0.1[e]	1.0	1.0	1.0	1.0	1.0	1.3	1.1	1.0	1.0	1.1	1.01	0.04	3.86	0.1	1/19
18:4n-3	2.0	1.9	2.0	1.9	2.0	2.0	1.9	1.9	2.0	2.0	2.0	1.9	2.0	1.9	1.9	2.0	4.3	2.1	2.0	2.2	1.9	1.97	0.08	4.24	0.2	0/19
20:0	0.1	—[f]	0.1	0.1	—	—	0.1	0.1	0.2	0.2	1.0	0.1	0.3	0.2	—	—	4.3[e]	0.3	0.1	4.4[e]	0.1	0.15	0.08	53.89	0.2	2/15
20:1	10.7	9.4	10.1	9.5	10.9	10.1	10.3	9.7	10.2	9.4	10.4	10.3	9.9	9.9	8.0	10.1	1.2[e]	10.3	9.5	4.4[e]	10.3	10.04	0.45	4.46	1.3	2/19
20:2n-6	—	—	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	—	—	0.4	0.3	0.2	0.5	0.3	0.31	0.08	25.77	0.2	0/15
20:3n-6	—	—	0.1	0.1	0.2	—	—	0.1	—	0.1	0.1	—	—	0.1	—	—	0.5	0.2	—	0.1	0.1	0.09	0.07	67.73	0.2	0/12
20:3n-3	0.1	—	0.1	0.1	—	0.2	0.2	0.2	—	0.1	0.1	—	0.1	0.1	0.1	0.5[e]	0.3	0.3	0.1	0.2	0.1	0.14	0.06	40.34	0.2	1/15
20:4n-6	0.4	—	0.5	0.4	0.8	0.5	0.5	0.5	0.4	0.5	—	0.5	0.4	0.4	0.4	—	0.6	0.4	0.4	0.6	0.4	0.42	0.19	43.83	0.5	0/18
20:4n-3	0.6	—	0.6	0.6	0.8	0.7	0.6	0.6	0.5	0.6	0.7	0.6	0.6	0.6	0.5	0.6	—	0.4	0.6	1.0[e]	0.8	0.59	0.08	13.76	0.2	1/18
20:5n-3	8.1	7.8	7.9	7.6	8.4	7.9	8.2	7.4	8.1	7.1	8.0	7.7	7.8	7.6	6.8	8.4	7.4	5.3[e]	7.5	7.4	7.9	7.76	0.43	5.48	1.2	1/19
22:0	1.2[e]	—	0.2	0.3	—	—	0.4	0.4	0.3	0.3	—	0.3	—	0.3	—	0.3	1.4[e]	—	0.3	—	—	0.32	0.03	11.77	0.1	2/12
22:1	8.8	7.7	9.4	9.1	10.2	9.3	9.9	8.8	9.5	8.6	9.9	9.4	9.3	9.4	7.2	8.8	7.2	7.6	8.4	9.1	8.8	8.96	0.80	8.88	2.2	0/19
22:4n-6	—	—	0.1	0.5	0.3	0.3	0.2	0.2	—	0.1	—	—	—	0.1	—	0.3	0.5	—	0.1	0.3	—	0.21	0.16	67.99	0.4	0/12
22:5n-6	—	—	0.1	0.2	0.2	0.1	0.2	0.1	—	0.1	0.1	—	—	0.1	—	—	—	—	0.1	0.1	—	0.13	0.04	31.75	0.1	0/11
22:5n-3	1.1	—	1.0	1.0	1.2	1.0	1.1	1.0	1.0	0.9	1.1	1.0	0.9	1.0	0.8	1.1	—	0.5[e]	0.9	1.1	1.0	1.01	0.10	9.73	0.3	1/18
22:6n-3	7.7	6.9	6.9	7.2	8.5	7.0	7.4	6.4	7.4	6.5	7.5	6.9	6.8	6.5	5.4	7.2	7.6	3.9[e]	6.0	7.4	7.4	6.97	0.69	9.86	1.9	1/19
24:0	—	—	—	—	—	—	0.3	0.3	0.3	0.4	0.4	—	—	0.1	—	—	0.5	—	—	—	0.9	0.24	0.14	59.08	0.4	0/07
24:1	2.6	—	0.3	—	0.8	0.4	0.8	0.7	0.7	0.5	1.4	1.2	—	0.6	0.4	2.5	1.1	—	0.6	0.5	0.6	0.93	0.73	78.73	2.0	0/15
mg/g sample																										
20:5n-3	57.0	61.2	65.2	66.0	63.7	63.4	63.3	65.1	62.0	66.9	70.0	118.7[e]	54.8	61.1	76.9	62.0	54.3	—	68.5	65.0	—	64.27	4.98	7.72	13.9	1/18
22:6n-3	53.0	53.2	56.3	61.9	62.4	54.6	56.6	56.2	55.0	60.1	63.0	104.4[e]	47.1	51.0	59.2	52.0	54.2	—	53.4	63.3	—	56.37	4.68	8.30	13.1	1/18

[a] Polyglycol column not used. Data are not included in statistical calculations.
[b] Reproducibility standard deviation.
[c] Reproducibility relative standard deviation.
[d] Reproducibility value ($2.8 \times s_R$).
[e] Outlier value, determined by Dixon and/or Grubbs tests, not used in statistical calculations.
[f] Denotes a value reported as "—," "< 0.05," "0," or "tr."

Table 5. Collaborative study results of analysis of Sample 4 (MaxEPA®)

	Composition, area %																									
	Laboratory																									
Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17[a]	18	19	20	21[a]	Mean	s_R[b]	RSD_R, %[c]	R[d]	Outliers/ No. labs.
14:0	6.5	7.3	7.4	7.7	5.6	6.8	6.2	7.0	6.7	7.5	6.6	6.8	7.6	6.8	10.1[e]	6.9	5.1	6.9	8.0	7.1	6.8	6.97	0.58	8.31	6.6	1/19
16:0	14.2	15.7	16.1	16.1	12.9	14.9	14.3	15.2	15.1	16.3	12.6	15.0	15.6	14.7	15.8	15.0	12.0	15.4	16.4	14.6	15.3	15.04	1.03	0.85	2.9	0/19
16:1	7.6	8.2	8.3	8.5	6.7	7.8	7.8	8.8	7.7	8.4	7.9	7.7	8.2	8.2	8.3	11.2[e]	9.2	9.1	8.5	7.6	7.2	8.06	0.55	6.80	0.5	1/19
18:0	3.0	3.2	3.1	2.8	3.1	3.0	1.8	3.0	1.8	3.2	3.4	3.1	2.9	3.0	2.7	3.1	2.6	3.2	3.0	2.8	3.0	2.90	0.42	14.43	1.2	0/19
18:1	13.8	14.5	14.6	14.1	13.8	13.9	14.1	14.1	14.1	14.3	13.9	14.0	14.1	14.2	13.3	14.4	10.4	14.8	14.1	13.0	13.9	14.06	0.42	2.98	1.2	0/19
18:2n-6	1.1	1.2	1.2	1.1	1.1	1.2	1.2	1.2	1.1	1.2	1.3	1.1	1.2	1.2	1.9	1.2	5.5[e]	1.2	1.2	1.1	1.2	1.16	0.05	4.65	0.2	1/19
18:3n-3	0.7	0.7	0.7	0.6	0.7	0.7	0.7	0.7	0.6	0.7	0.5	0.7	0.7	0.7	0.6	0.7	1.2	0.8	0.6	0.7	0.7	0.66	0.06	9.16	0.2	0/19
18:4n-3	2.1	2.1	2.2	2.0	2.0	2.1	2.1	2.0	2.0	2.1	2.3	1.9	2.1	2.0	1.8	2.1	3.3	2.4	2.1	2.0	2.0	2.07	0.13	6.30	0.4	0/19
20:0	0.4	—[f]	0.4	0.4	—	—	0.4	0.4	0.4	0.4	0.8	0.4	0.3	0.4	—	0.4	2.7	0.5	0.3	1.0	0.4	0.47	0.17	37.24	0.5	0/15
20:1	3.3	3.1	3.1	2.9	4.2	3.2	3.3	3.1	3.0	3.1	3.7	3.3	3.1	3.2	0.5[e]	3.5	1.2	3.5	3.0	1.9[e]	3.1	3.25	0.32	9.70	0.9	2/19
20:2n-6	0.2	—	0.2	0.2	0.2	0.2	0.2	0.2	—	0.2	0.2	0.2	0.2	0.1	0.1	—	0.5	0.2	0.1	0.2	0.2	0.16	0.03	21.35	0.1	0/16
20:3n-6	0.1	—	0.1	0.1	0.3	—	0.1	0.1	—	0.1	0.1	0.1	0.1	0.1	—	—	1.5	0.2	0.1	0.2	0.1	0.13	0.07	50.84	0.2	0/15
20:3n-3	0.1	—	0.1	0.1	0.2	—	0.1	0.3	—	0.1	0.1	—	0.1	0.1	0.1	0.9[e]	—	0.3	0.1	0.1	0.1	0.15	0.07	47.93	0.2	1/15
20:4n-6	0.8	0.9	0.8	0.8	1.0	0.8	0.9	0.9	0.8	0.8	1.0	0.8	0.8	0.8	0.7	—	0.4[e]	0.9	0.8	0.8	0.8	0.83	0.07	8.86	0.2	1/19
20:4n-3	0.8	0.8	0.8	0.8	0.9	0.9	0.8	0.8	0.8	0.8	1.0	0.8	0.7	0.8	0.6	0.8	—	0.9	0.7	0.7	0.8	0.80	0.08	10.44	0.2	0/19
20:5n-3	17.9	17.8	17.2	16.9	18.4	17.4	17.5	16.3	18.1	15.6	15.8	16.8	17.3	16.8	14.4	17.5	16.4	19.9	16.2	15.2	17.4	16.99	1.26	7.42	3.5	0/19
22:0	—	—	—	0.2	—	—	0.1	0.1	—	0.1	0.1	0.1	0.1	0.1	—	—	1.2[e]	—	0.1	—	0.1	0.11	0.02	15.93	0.1	1/10
22:1	2.5	2.5	2.4	2.4	2.8	2.5	2.7	2.4	2.5	2.3	3.0	2.8	2.3	2.3	2.0	2.1	3.1	2.4	2.3	2.2	2.6	2.44	0.24	10.02	0.7	0/19
22:4n-6	—	—	0.3	0.2	0.3	—	0.1	0.1	0.1	0.3	0.1	0.1	0.3	0.1	—	—	0.8	—	0.1	0.2	0.1	0.16	0.09	56.22	0.3	0/14
22:5n-6	0.3	—	—	0.3	0.4	0.3	0.3	0.3	0.3	0.1[e]	0.3	0.3	—	0.3	—	0.6[e]	—	—	0.2	1.4[e]	—	0.29	0.04	13.14	0.1	3/14
22:5n-3	2.3	2.2	2.0	1.8	2.3	2.1	2.2	2.0	2.2	1.8	2.5	2.2	1.9	2.0	1.5	2.1	—	2.1	1.8	2.5	2.1	2.07	0.25	12.13	0.7	0/19
22:6n-3	13.5	12.6	11.9	12.1	13.4	12.3	12.5	11.2	13.3	10.6	12.4	12.4	12.0	11.4	8.8	12.6	12.3	15.1	10.5	14.5	12.8	12.27	1.44	11.74	4.0	0/19
24:0	—	—	—	—	—	—	0.1	—	0.1	0.1	—	0.1	—	0.1	—	—	—	—	0.1	—	0.1	0.06	0.07	70.06	0.2	0/07
24:1	0.6	—	—	—	0.4	—	0.6	0.5	0.6	—	—	0.7	—	0.5	—	0.5	0.7	—	0.5	—	0.6	0.44	0.23	52.96	0.7	0/11
	mg/g sample																									
20:5n-3	135.0	144.4	148.8	158.6	125.7	141.0	143.8	153.6	137.0	152.2	144.0	244.2	154.3	145.8	232.6	170.0	131.7	—	156.0	147.2	—	157.46	31.10	9.75	87.1	0/18
22:6n-3	100.0	100.0	101.0	112.4	88.0	98.2	101.4	105.2	99.0	101.9	104.0	176.2[e]	104.8	97.0	138.9	102.0	97.1	—	99.0	137.3	—	105.30	13.27	2.60	37.1	1/18

[a] Polyglycol column not used. Data are not included in statistical calculations.
[b] Reproducibility standard deviation.
[c] Reproducibility relative standard deviation.
[d] Reproducibility value ($2.8 \times s_R$).
[e] Outlier value, determined by Dixon and/or Grubbs tests, not used in statistical calculations.
[f] Denotes a value reported as "—," "< 0.05," "0," or "tr."

Table 6. Collaborative study results of analysis of Sample 5 (Promega®)

Fatty acids	Composition, area % — Laboratory 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17[d]	18	19	20	21[e]	Mean	s_R[b]	RSD_R, %[c]	R[d]	Outliers/ No. labs.
14:0	4.9	5.5	5.5	5.8	4.3	4.7	4.7	5.3	4.7	5.6	5.5	5.6	5.7	5.1	7.2	5.3	4.8	10.8[e]	5.9	5.5	5.1	5.37	0.64	11.98	1.8	1/19
16:0	7.2	8.0	8.2	8.0	6.7	7.4	7.5	7.8	7.1	8.4	7.9	8.2	8.2	7.6	7.9	7.8	6.0	13.7[e]	8.2	7.9	7.8	7.77	0.44	5.66	1.2	1/19
16:1	8.4	8.6	9.3	9.5	7.5	8.2	8.5	9.9	7.6	9.5	8.9	9.1	9.3	8.4	9.1	17.0	9.6	16.6	9.4	9.0	8.3	9.67	2.59	26.73	7.2	0/19
18:0	0.8	4.2[e]	0.8	0.8	0.8	0.8	0.9	0.8	0.7	0.9	0.9	0.9	0.8	0.8	0.8	0.9	0.8	7.3[e]	0.8	0.9	0.8	0.82	0.05	5.56	0.1	2/19
18:1	9.6	10.3	10.3	9.8	10.8	9.8	9.9	10.1	9.2	10.3	10.4	10.3	10.0	9.9	9.3	10.3	9.2	11.5	10.0	9.8	9.8	10.17	0.68	6.71	1.9	0/19
18:2n-6	1.1	1.2	1.2	1.1	1.2	1.1	1.2	1.2	1.0	1.2	1.3	1.2	1.1	1.1	1.1	1.2	3.2	1.3	1.2	1.1	1.2	1.16	0.07	5.97	0.2	0/19
18:3n-3	0.9	1.0	1.0	0.9	0.9	1.0	1.0	1.0	0.8	1.0	0.2	1.0	0.9	0.9	0.8	1.0	6.6[e]	1.0	1.0	0.9	1.0	0.94	0.06	5.98	0.2	1/19
18:4n-3	4.4	4.6	0.2[e]	4.3	4.3	4.4	4.5	4.3	4.0	4.5	4.8	4.3	4.5	4.4	4.0	4.5	0.4	5.0	4.5	4.3	4.4	4.42	0.24	5.45	0.7	1/19
20:0	0.1	—[f]	0.1	0.5	—	—	0.1	0.1	—	0.2	1.1	0.1	0.2	0.2	—	—	1.7[e]	0.2	0.1	0.6	0.1	0.19	0.16	84.25	0.4	1/14
20:1	1.5	1.4	1.5	1.4	1.2	1.5	1.5	1.4	1.2	1.5	1.6	1.5	1.3	1.5	1.2	0.7	0.3	1.0	1.4	0.9	1.5	1.32	0.24	18.13	0.7	0/19
20:2n-6	0.1	—	0.1	0.1	0.1	0.1	0.1	0.1	—	0.1	0.1	—	—	0.1	—	—	—	—	0.1	0.1	0.1	0.10	0.01	10.29	0.1	0/14
20:3n-6	0.1	—	0.1	0.2	0.4[e]	—	0.2	0.2	—	0.1	0.1	—	0.1	0.1	—	0.2	0.3	0.1	0.1	0.3	0.2	0.14	0.05	34.18	0.1	1/14
20:3n-3	—	—	0.1	0.2	0.2	0.2	0.1	0.1	—	0.1	0.1	—	—	0.1	—	0.8[e]	0.9[e]	0.2	0.1	0.2	0.1	0.14	0.04	27.47	0.1	2/14
20:4n-6	0.8	1.2[e]	0.8	0.7	0.8	0.8	0.8	0.8	0.9	0.8	0.9	0.8	0.7	0.7	0.6	—	—	0.6	0.7	0.7	0.8	0.75	0.08	10.85	0.2	2/19
20:4n-3	1.1	0.8	1.1	1.0	1.2	1.2	1.2	1.1	1.0	1.2	1.4[e]	1.2	1.1	1.1	0.9	1.2	—	0.8	1.2	1.1	1.2	1.09	0.15	13.77	0.4	1/19
20:5n-3	29.3	29.4	28.7	27.8	29.9	28.8	28.6	27.1	27.8	26.8	24.1	28.0	28.7	28.4	24.9	29.1	27.0	21.6	27.3	26.8	29.1	27.54	2.06	7.49	5.8	0/19
22:0	—	—	—	0.1	—	—	—	—	—	—	1.1	—	—	0.1	—	—	1.4[e]	—	—	—	—	0.03	0.38	141.42	1.1	1/06
22:1	0.9	0.9	1.0	1.1	1.2	1.1	1.1	1.1	0.8	1.0	1.2	0.9	0.9	1.0	1.3	0.9	1.7	—	0.8	1.1	1.1	1.02	0.14	13.35	0.04	0/18
22:4n-6	—	—	—	0.1	0.1	—	—	0.1	0.4	0.2	—	—	—	0.1	—	0.9[e]	0.9	—	0.1	—	—	0.13	0.14	93.55	0.4	1/09
22:5n-6	0.2	—	—	0.2	0.2	0.2	0.2	0.3	0.5	0.1[e]	0.2	—	0.2	0.2	0.2	—	—	—	0.1	1.2[e]	—	0.20	0.07	28.69	0.2	2/14
22:5n-3	2.4	2.3	2.2	2.0	2.4	2.3	2.3	2.1	2.1	2.0	2.5	2.3	2.1	2.2	1.7	2.3	—	1.2[e]	2.0	2.0	2.3	2.18	0.19	8.84	0.5	1/19
22:6n-3	14.1	13.0	12.3	12.2	13.5	13.1	13.0	12.0	12.6	11.2	12.9	12.7	12.5	12.5	10.1	13.3	13.3	7.1[e]	11.7	11.4	13.8	12.45	0.93	7.50	2.6	1/19
24:0	—	—	—	—	—	—	0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.00	0.02	100.00	0.1	1/05
24:1	0.2	—	—	—	0.3	—	0.6	0.2	0.2	—	—	0.2	—	0.1	—	—	0.4	—	—	—	0.2	0.17	0.07	102.20	0.2	0/10
mg/g sample																										
20:5n-3	232.0	240.4	253.7	262.9	245.8	235.0	242.7	250.8	234.0	262.4	213.0	412.9[e]	236.6	239.4	228.4	240.6	214.6	—	256.0	230.0	—	241.40	12.98	5.38	36.3	1/18
22:6n-3	109.0	106.4	106.7	114.8	106.5	105.0	109.1	110.9	104.0	107.5	107.0	183.4[e]	100.6	102.8	91.0	108.0	103.5	—	107.0	96.3	—	105.44	5.52	5.23	15.5	1/18

[a] Polyglycol column not used. Data are not included in statistical calculations.
[b] Reproducibility standard deviation.
[c] Reproducibility relative standard deviation.
[d] Reproducibility value ($2.8 \times s_R$).
[e] Outlier value, determined by Dixon and/or Grubbs tests, not used in statistical calculations.
[f] Denotes a value reported as "—," "<0.05," "0," or "tr."

Table 7. Collaborative study results of analysis of Sample 6 (blind duplicate, steam-deodorized menhaden oil)

	Composition, area %																									
	Laboratory																									
Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17[a]	18	19	20	21[a]	Mean	s_R[b]	RSD_R, %[c]	R[d]	Outliers/ No. labs.
14:0	8.1	7.1	8.1	10.9	6.7	7.9	7.4	8.0	7.6	8.3	7.7	8.8	8.7	8.8	13.4[e]	8.2	6.0	9.6	9.3	8.2	7.8	8.30	0.96	11.62	2.7	1/19
16:0	18.4	17.9	19.3	21.8	16.5	18.6	18.0	18.5	19.7	19.6	15.2	19.9	19.3	19.2	18.9	19.1	15.3	24.5	19.9	17.8	19.1	19.05	1.91	10.02	5.3	0/19
16:1	11.7	10.9	11.7	13.6	10.0	11.3	11.3	12.4	11.3	11.9	10.8	11.4	11.9	11.7	11.9	13.8	12.9	15.6	12.4	10.9	10.9	11.91	1.27	10.66	3.5	0/19
18:0	3.1	3.2	3.2	2.7	3.1	3.7	3.1	3.0	3.1	3.2	3.5	3.2	3.0	3.0	2.3[e]	3.3	2.6	3.8[e]	3.0	2.8	3.1	3.09	0.18	5.71	0.5	2/19
18:1	11.9	12.2	12.3	11.2	11.9	11.7	11.9	11.8	12.1	12.0	12.4	12.3	11.9	11.7	9.7[e]	12.5	12.2	14.6[e]	11.5	10.8	11.9	11.88	0.43	3.62	1.2	2/19
18:2n-6	1.2	1.2	1.4	1.1	1.2	1.2	1.2	1.2	1.1	1.2	1.3	1.2	1.2	1.2	0.9	1.2	2.3	1.5	1.1	1.1	1.1	1.20	0.13	10.69	0.4	0/19
18:3n-3	0.8	0.8	0.7	0.7	0.8	0.8	0.8	0.8	0.7	0.8	0.3	0.8	0.4	0.7	0.6	0.8	6.0	1.0	0.8	1.0	0.8	0.75	0.17	23.14	0.5	0/19
18:4n-3	3.0	3.0	3.0	2.7	2.9	2.9	2.9	2.9	2.9	2.9	3.3	2.8	2.9	2.8	2.4	3.1	0.5[e]	3.5[e]	2.9	2.9	2.8	2.92	0.13	4.48	0.4	2/19
20:0	0.2	—[f]	0.2	0.2	—	—	0.2	0.2	0.2	0.2	0.9[e]	0.2	0.2	0.2	—	0.2	2.3[e]	0.3	0.2	—	0.2	0.21	0.02	8.52	0 1	2/14
20:1	1.7	1.8	2.0	1.4	2.5	1.9	1.9	1.9	1.9	1.6	2.1	1.6	1.7	1.8	1.2	2.0	0.5	1.5	1.5	2.2	1.9	1.80	0.29	16.24	0.8	0/19
20:2n-6	0.2	—	0.2	0.1	0.2	0.2	0.2	0.2	—	0.2	0.1	0.1	0.1	0.1	—	—	0.4	—	0.1	0.2	0.2	0.15	0.04	25.41	0.1	0/14
20:3n-6	0.2	—	0.2	0.3[e]	0.4[e]	0.2	0.2	0.2	—	0.2	—	0.1	0.2	0.2	—	0.2	0.4	—	0.2	0.2	0.2	0.19	0.03	16.08	0.1	2/15
20:3n-3	—	—	0.1	0.2	0.3	0.2	0.2	0.2	—	0.1	0.2	—	0.1	0.1	—	0.9[e]	1.0[e]	0.2	0.1	0.4	0.1	0.16	0.05	28.55	0.1	2/14
20:4n-6	0.8	1.0	0.3[e]	0.7	1.0	0.9	0.9	0.8	0.8	0.8	1.0	0.9	0.8	0.8	0.6	—	0.6	0.9	0.8	1.0	0.8	0.84	0.10	12.08	0.3	2/19
20:4n-3	1.3	1.5	1.3	1.1	1.4	1.3	1.3	1.3	1.3	1.3	1.5	1.3	1.2	1.2	0.8[e]	1.3	—	1.3	1.2	2.1[e]	1.4	1.29	0.11	8.17	0.3	2/19
20:5n-3	14.6	15.3	14.0	11.4	14.9	13.6	13.9	12.9	14.8	12.4	13.7	13.5	13.7	13.2	9.7	14.2	14.0	14.0	12.7	12.6	14.1	13.42	1.31	9.78	3.7	0/19
22:0	—	—	0.3	0.2	—	—	0.2	0.2	—	0.1	0.1	—	0.1	0.2	—	—	0.6	—	0.2	—	0.1	0.17	0.08	48.00	0.2	0/10
22:1	0.9	1.6	1.5	1.2	1.6	1.3	1.4	1.3	1.1	1.4	1.7	1.2	1.6	1.4	1.2	1.3	1.2	—	1.1	3.0[e]	0.9	1.35	0.22	16.05	0.6	1/18
22:4n-6	—	—	0.1	0.3	0.3	0.2	0.2	0.3	—	0.2	0.1	—	0.2	0.2	—	0.7	0.9[e]	—	0.1	0.2	0.1	0.20	0.08	38.29	0.2	1/13
22:5n-6	0.2	—	0.1	0.2	0.2	0.2	0.2	0.2	—	0.1	0.2	—	—	0.1	0.1	—	—	—	0.1	0.3	—	0.16	0.08	50.60	0.2	0/14
22:5n-3	2.3	2.4	2.0	1.5	2.3	2.1	2.1	1.9	2.2	1.8	2.3	2.1	1.9	1.9	1.2	2.2	—	1.5	1.8	1.9	2.2	1.97	0.32	16.20	0.9	0/19
22:6n-3	9.3	9.5	8.1	6.2	9.1	7.9	8.2	7.5	9.1	7.1	8.7	8.0	7.9	7.6	4.8	8.6	9.4	6.2	6.7	7.5	8.7	7.77	1.20	15.48	3.4	0\19
24:0	—	—	—	—	—	—	0.1	0.1	0.3	0.1	0.1	—	—	0.1	0.2	—	—	—	—	—	0.3	0.14	0.07	48.38	0.2	0/06
24:1	—	—	0.1	—	0.4	0.2	0.4	0.4	0.4	0.2	0.5	0.6	—	0.4	—	0.6	0.6	—	—	0.4	0.3	0.36	0.14	41.22	0.4	0/13
	mg/g sample																									
20:5n-3	124.0	108.8	120.2	133.1	135.8	116.0	116.5	118.2	107.0	119.7	118.0	230.6[e]	103.2	102.7	121.9	112.0	105.0	—	126.0	118.3	—	116.54	9.25	7.86	25.9	1/18
22:6n-3	77.0	67.6	67.6	71.3	79.6	65.5	67.8	68.3	65.0	67.5	70.0	134.2[e]	57.8	57.7	59.5	66.0	69.0	—	65.5	69.2	—	67.23	5.76	8.57	16.1	1/18

[a] Polyglycol column not used. Data are not included in statistical calculations.
[b] Reproducibility standard deviation.
[c] Reproducibility relative standard deviation.
[d] Reproducibility value ($2.8 \times s_R$).
[e] Outlier value, determined by Dixon and/or Grubbs tests, not used in statistical calculations.
[f] Denotes a value reported as "—," "< 0.05," "0," or "tr."

Table 8. Statistical evaluation of collaborative study of a marine oil and blind duplicate on capillary GC column coated with a bonded polyglycol liquid phase[a]

Fatty acid	Composition, area %							Outliers/ No. laboratories
	Mean	s_R	RSD_R, %	R	s_r	RSD_r, %	r	
14:0	8.27	0.95	11.26	2.7	0.49	5.79	1.4	1/19
16:0	18.72	1.36	7.27	3.9	0.54	2.89	1.5	1/19
16:1	11.99	1.47	12.26	4.2	0.51	4.28	1.5	0/19
18:0	3.10	0.15	4.76	0.4	0.06	1.84	0.2	2/19
18:1	11.90	0.34	2.88	1.0	0.19	1.57	0.5	2/19
18:2n-6	1.19	0.06	4.65	0.2	0.01	1.19	0.0	4/19
18:3n-3	0.75	0.17	22.06	0.5	0.04	5.58	0.1	0/19
18:4n-3	2.91	0.08	2.85	0.2	0.04	1.52	0.1	3/19
20:0	0.21	0.02	8.07	0.1	0.01	5.46	0.0	2/14
20:1	1.81	0.27	14.98	0.8	0.13	7.27	0.4	0/19
20:2n-6	0.15	0.04	25.87	0.1	0.01	8.64	0.0	1/14
20:3n-6	0.19	0.04	21.53	0.1	0.03	17.35	0.1	2/16
20:3n-3	0.16	0.06	30.63	0.2	0.01	3.11	0.0	3/15
20:4n-6	0.84	0.09	11.62	0.3	0.02	2.53	0.1	3/19
20:4n-3	1.30	0.10	7.74	0.3	0.04	3.46	0.1	3/19
20:5n-3	13.80	0.80	5.93	2.3	0.25	1.85	0.7	2/19
22:0	0.18	0.11	59.75	0.3	0.02	8.92	0.0	0/12
22:1	1.37	0.23	16.27	0.6	0.11	7.86	0.3	2/18
22:4n-6	0.19	0.11	49.94	0.3	0.06	26.34	0.2	1/14
22:5n-6	0.16	0.06	48.89	0.2	0.03	18.61	0.1	0/15
22:5n-3	1.97	0.37	18.99	1.1	0.13	6.72	0.4	0/19
22:6n-3	7.87	1.23	15.74	3.5	0.29	3.72	0.8	1/19
24:0	0.12	0.07	61.03	0.2	0.01	10.93	0.0	0/07
24:1	0.32	0.15	44.16	0.4	0.03	7.37	0.1	2/16
	mg/g sample							
20:5n-3	116.54	8.56	7.03	24.2	7.17	5.89	20.3	1/18
22:6n-3	66.61	4.74	6.80	13.4	3.68	5.27	10.4	1/18

[a] s_R = reproducibility standard deviation, RSD_R = reproducibility relative standard deviation; R = reproducibility value ($2.8 \times s_R$); s_r = repeatability standard deviation; RSD_r = repeatability relative standard deviation; r = repeatability value ($2.8 \times s_r$).

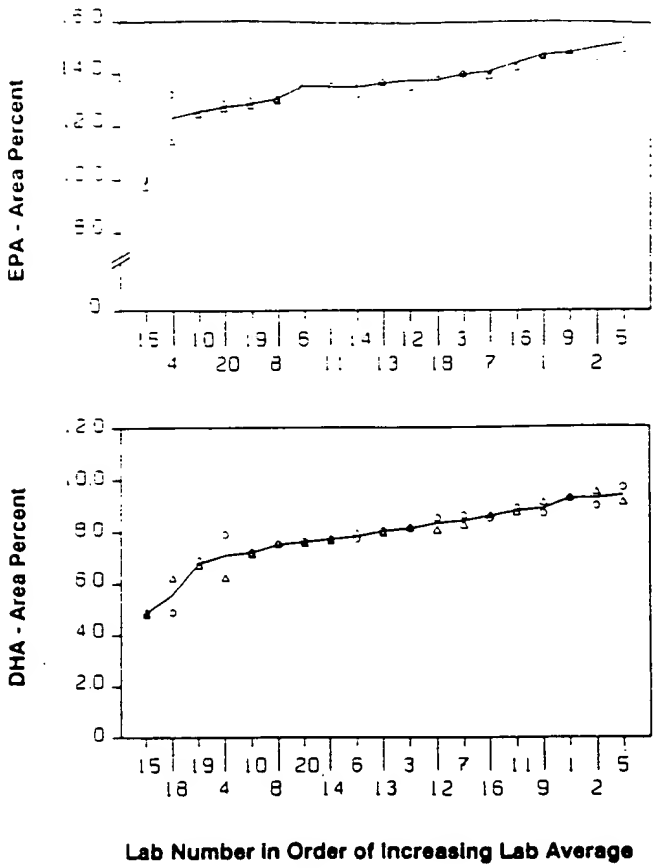

Figure 1. GC analysis of EPA and DHA in Samples 1 (o) and 6 (Δ) (blind duplicate): concentration in area percent vs collaborator number in ascending order.

orator responded that the internal standard had been improperly prepared and requested that the data be disregarded. Collaborator 12 originally reported weights of EPA and DHA ranging from 2800 to 10 000 mg/g in the 6 samples, clearly reflecting errors in the calculations. Subsequently, recalculated values were submitted and are listed in Tables 2–7.

The results of between-laboratory reproducibility (RSD_R) and within-laboratory repeatability (RSD_r) calculations derived from the analysis of Sample 1 and its blind duplicate, Sample 6, are given in Table 8. The ranges and, hence, the variabilities in the determination of the area percentages of EPA and DHA are typified in Figure 1 and those for the absolute weights in Figure 2.

All calculations except the reproducibility values (R) and repeatability values (r) were performed by using the computer program FDACHEMIST, which was developed for the statistical analysis of collaborative study data (2). The identities (but not the existence) of Dixon and/or Grubbs between-laboratory outlier values were determined by visual inspection, referred to by Albert as the "ultimate outlier test" (3), and are indicated in Tables 2–7. The Cochran test was used to calculate within-laboratory outlier values, also by using FDACHEMIST.

A summary of the statistical performance of the method for all of the 26 analytes in the 5 oils and the ethyl ester concentrate is given in the method as Method Performance. Because of the

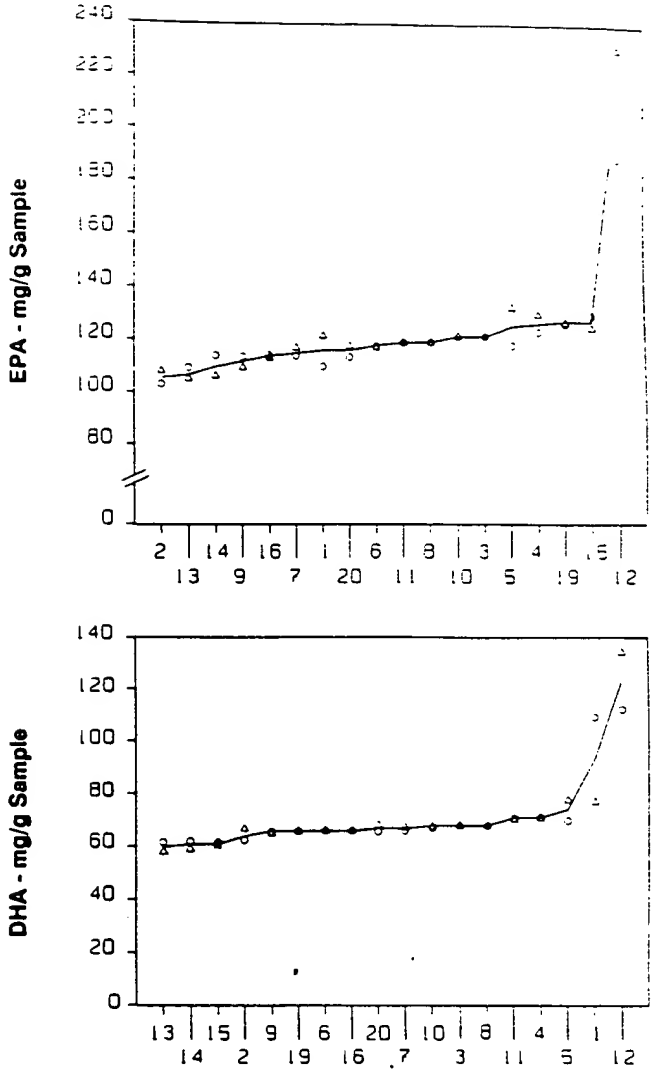

Figure 2. GC analysis of EPA and DHA in Samples 1 (o) and 6 (Δ) (blind duplicate): concentration in mg/g sample vs collaborator number in ascending order.

large amount of data generated in the analysis of the oils, ranges for s_R and RSD_R, rather than individual values, are listed for this matrix. Repeatability standard deviations (s_r and RSD_r) could be calculated only for the analysis of one oil and its blind duplicate.

Collaborators' Comments

Collaborator 6 noted that a labeled chromatogram of fish oil ethyl esters would have aided in identification of the components in Sample 2.

Using a DB-Wax column, Collaborator 11 observed coelution of some sample components with the internal standard; correction was made for this coelution. This collaborator also commented that when their chromatographic system is "functioning properly" (phrase not defined), it gives response factors that are within ±1% of the theoretical response factors published by Craske and Bannon (4). When it is not functioning

Table 9. Comparison of 1977 AOCS Smalley, 1979 AOAC, and 1988 AOAC collaborative study results

Fatty Acid	14:0	16:0	16:1	18:0	18:1	18:2	20:1	20:5	22:1	22:6
1977 AOCS										
N	55	53	55	55	55	51	55	41	52	33
Mean, %	4.2	8.6	13.1	1.2	13.7	1.2	18.8	6.6	21.7	3.2
SD	0.60	0.60	1.28	0.51	0.67	0.63	1.25	0.66	2.87	0.42
CV, %	14.3	6.9	9.8	28.2	4.9	53.4	6.6	10.0	13.2	13.1
1979 AOAC										
N	15	14	14	15	15	15	14	13	13	14
Mean, %	4.1	8.7	13.2	1.9	13.9	0.9	18.7	7.0	21.5	3.5
SD	0.44	0.69	0.97	0.39	0.77	0.41	0.72	0.59	1.29	0.69
CV, %	10.7	7.9	7.4	20.6	5.6	45.1	3.9	8.5	6.0	19.9
1988 AOAC										
Sample 1										
N	19	18	19	17	17	18	17	18	17	19
Mean, %	8.3	18.7	12.1	3.1	11.9	1.2	1.8	13.8	1.4	7.9
SD	0.98	1.31	1.65	0.11	0.23	0.07	0.15	0.79	0.25	1.27
CV, %	11.7	7.0	13.6	3.6	1.9	5.6	8.0	5.8	17.2	16.1
Sample 2										
N	17	17	15	17	17	17	17	18	17	18
Mean, %	0.4	0.9	0.3	2.1	8.7	0.8	14.2	26.4	10.6	18.7
SD	0.06	0.11	0.10	0.15	0.50	0.06	0.45	1.54	0.50	1.40
CV, %	17.1	12.2	31.0	7.0	5.7	7.9	3.2	5.8	4.7	7.5
Sample 3										
N	19	18	18	18	18	18	17	18	19	18
Mean, %	5.7	13.3	8.8	2.3	18.0	2.0	10.0	7.8	9.0	7.0
SD	0.75	1.00	0.83	0.07	0.51	0.05	0.45	0.43	0.80	0.69
CV, %	13.1	7.5	9.4	3.1	2.8	2.4	4.5	5.5	8.9	9.9
Sample 4										
N	18	19	18	19	19	18	17	19	19	19
Mean, %	7.0	15.0	8.1	2.9	14.1	1.2	3.3	17.0	2.4	12.3
SD	0.58	1.03	0.55	0.42	0.42	0.05	0.32	1.26	0.24	1.44
CV, %	8.3	6.9	6.9	14.4	3.0	4.7	9.7	7.4	10.1	11.7
Sample 5										
N	17	18	19	17	19	18	19	19	18	18
Mean, %	5.4	7.8	9.7	0.8	10.1	1.2	1.3	27.5	1.0	12.5
SD	0.64	0.44	2.59	0.05	0.52	0.07	0.24	2.06	0.14	0.93
CV, %	12.0	5.7	26.7	5.6	5.2	6.0	18.1	7.5	13.4	7.5
Sample 6										
N	18	19	19	17	17	19	19	19	18	19
Mean, %	8.3	19.1	11.9	3.1	11.9	1.2	1.8	13.4	1.4	7.8
SD	0.96	1.91	1.27	0.18	0.42	0.13	0.29	1.31	0.22	1.20
CV, %	11.6	10.0	10.6	5.7	3.6	10.7	16.2	9.8	16.1	15.5

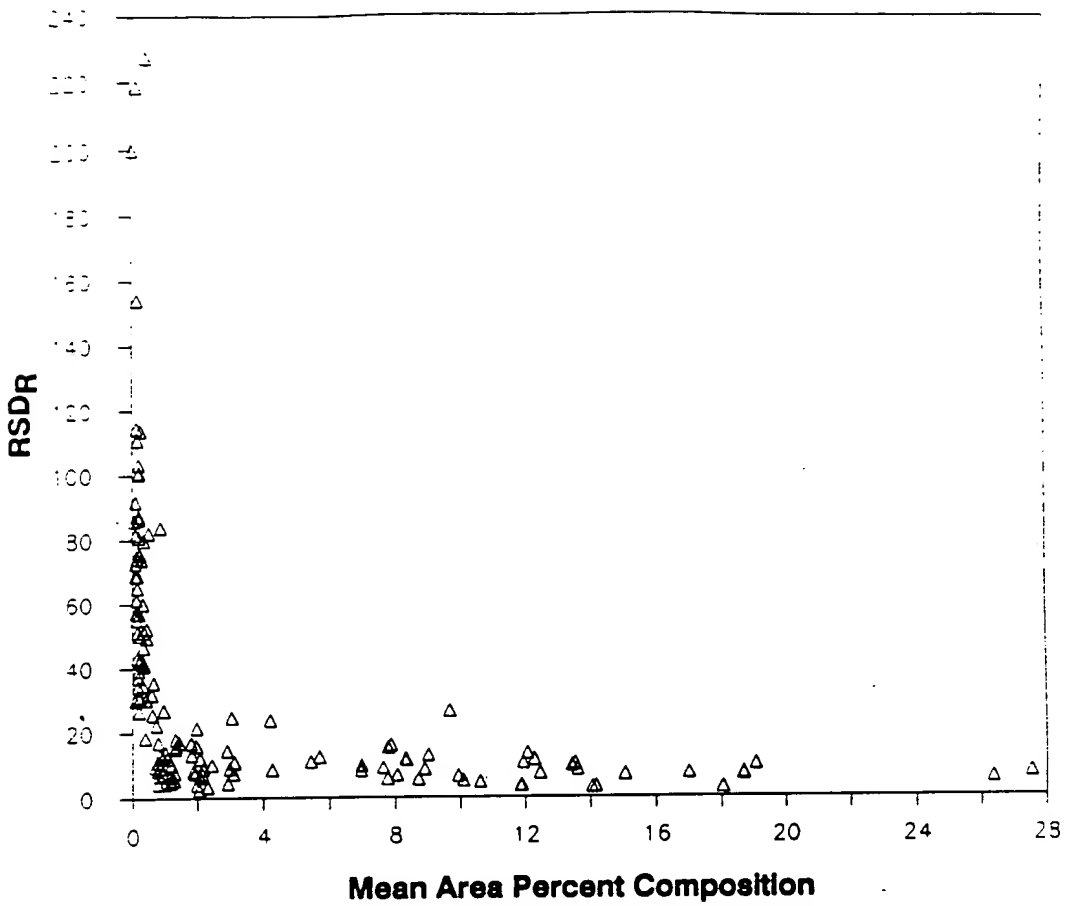

Figure 3. Reproducibility relative standard deviation (RSD_R) for determination of 24 analytes in 5 encapsulated fish oils and 1 ethyl ester concentrate as function of mean area percent composition.

properly, they find it almost impossible to obtain the theoretical factors. This occurred during the collaborative study for unknown reasons. They applied correction factors of 1.03 and 1.05 for EPA and DHA, respectively, to obtain the values reported for the study. This collaborator believes that one or more factors other than the column can affect the results of an analysis, but these factors were not identified.

Collaborator 14 submitted a secondary table of 16 additional analytes in the samples, in addition to the 24 components designated on the data sheet. In his view, the methylation procedure is unnecessarily complicated by the requirement for frequent nitrogen flushing because he believes the acids are unlikely to oxidize during their conversion to esters.

Collaborator 15 commented that the use of the suggested theoretical detector correction factors of 0.99 and 0.97 for EPA and DHA, respectively, gives substantially low values of these materials in fish oils and esters although these factors were used in preparing his report. This laboratory considers that more appropriate correction factors, relative to the internal standard (23:0), would be 0.80 for EPA and 0.68 for DHA. Another comment concerned the small volume of sample solution obtained. This presented a problem because this laboratory uses autosampler vials that require about 1.1 mL sample per injection.

Collaborator 17 raised 3 questions: (*1*) Why is there an apparent large difference between their area percent composition and their calculated weight composition? (*2*) Is 23:0 the most appropriate internal standard, given its relatively poor solubility in many organic solvents? (*3*) What are the relative merits of on-column vs split injection?

Discussion

Conventional GC on packed columns measures the proportions of individual fatty acids after their conversion from triacylglycerol form to volatile methyl ester form (5). However, this approach has several shortcomings. Packed columns are capable of resolving about 12 major or biochemically interesting fatty acids of fish oils (6), but fish oils contain 60 or more constituent fatty acids (7, 8). This limited resolving power of the polar packed GC columns formerly in common use may result in coelution of other components with EPA and/or DHA, exaggerating their respective percentages. This problem of peak coincidence was addressed earlier by Ackman when he described the chain length overlap that is common with packed polar columns (9). As an example, methyl docosenoate (22:1) coelutes with methyl arachidonate (20:4n-6) on the most highly

Table 10. Precision of capillary column analysis of encapsulated marine oil fatty acid esters

	Composition, area %													
	Sample[a]													
	1		2		3		4		5		6		1 and 6	
Fatty acids	Mean	RSD_R, %[b]	Mean	RSD_R, %	Mean	RSD_R, %	Mean	RSD_R, %	Mean	RSD_R, %	Mean	RSD_R, %	Mean	RSD_r, %[c]
14:0	8.3	11.7	0.4	17.1	5.7	13.1	7.0	8.3	5.4	12.0	8.3	11.6	8.3	5.8
16:0	18.7	7.0	0.9	12.2	13.3	7.5	15.0	6.9	7.8	5.7	19.0	10.0	18.7	2.9
16:1	12.1	13.6	0.3	31.0	8.8	7.5	8.1	6.8	9.7	26.7	11.9	10.7	12.0	4.3
18:0	3.1	3.6	2.1	7.0	2.3	3.1	2.9	14.4	0.8	5.6	3.1	5.7	3.1	1.8
18:1	11.9	1.9	8.7	5.7	18.0	2.8	14.1	3.0	10.2	6.7	11.9	3.6	11.9	1.6
18:4n-3	2.9	2.4	1.9	7.6	2.0	4.2	2.1	6.3	4.4	5.5	2.9	4.5	2.9	1.5
20:1	1.8	8.0	14.2	3.2	10.0	4.5	3.2	9.7	1.3	18.1	1.8	16.2	1.8	7.3
20:5n-3	13.8	5.8	26.4	5.6	7.8	5.5	17.0	7.4	27.5	7.5	13.4	9.8	13.8	1.9
22:1	1.4	17.2	10.6	4.7	9.0	8.9	2.4	10.0	1.0	13.4	1.4	6.1	1.4	7.9
22:5n-3	2.0	14.6	4.3	8.5	1.0	9.7	2.1	12.2	2.2	8.8	2.0	16.2	2.0	6.7
22:6n-3	7.9	16.1	18.7	7.5	7.0	9.9	12.3	11.7	12.5	7.5	7.8	15.5	7.9	3.7
	mg/g sample													
20:5n-3	115.3	6.0	223.1	9.2	64.3	7.7	157.5	19.8	241.4	5.4	117.7	7.9	116.5	5.9
22:6n-3	66.0	4.2	157.9	9.0	56.4	8.3	105.3	12.6	105.4	5.2	67.2	8.6	66.6	5.3

[a] Soft gelatin encapsulated samples: 1, steam-deodorized menhaden oil; 2, commercial ethyl ester preparation; 3, cod liver oil; 4, commercial fish oil; 5, commercial fish oil enriched in EPA and DHA; 6, steam-deodorized menhaden oil (blind duplicate of Sample 1).

[b] Reproducibility relative standard deviation.

[c] Repeatability relative standard deviation.

polar columns but with methyl EPA on less polar columns (6, 9). These particular GC problems can be overcome by the use of polar capillary columns, particularly the relatively low polarity polyglycol (Carbowax-20M) columns that have provided for nearly 2 decades maximum resolution of a large number of methyl esters of fatty acids without chain length overlap (10, 11). A typical chromatogram of menhaden oil fatty acid methyl esters on Carbowax-20M is illustrated in Figure 991.39.

Another problem in the determination of EPA and DHA in marine oils arises from the complex nature of the sample itself. In many analyses of fats and oils, it is conventional to regard the area percentages of fatty acid methyl esters as representative of the mass of the fatty acids in the oil sample; the glycerol content is generally ignored. However, unrefined fish oils contain at least 0.5% free sterols, mostly cholesterol in all instances (12), and, depending on origin, may also contain biogenic hydrocarbons, such as pristane or squalene (13), or fatty alcohols (14). Hexadecanol and docosenols are only 2 examples of the latter, which may be present at as much as 1% of the total lipid content (15). Although some of the more volatile impurities may be removed during steam deodorization, the final step in oil refining (16, 17), this does not include stearyl esters or wax esters (14), also commonly found in marine oils. Unfortunately, during deodorization, the labile EPA and DHA may form either thermal artifacts (18) or nonvolatile oxidative polymers (19). Moreover, companies producing encapsulated fish oils may add tocopherols and sometimes other materials as antioxidants or stabilizers (20). Because polymers, or other nonvolatile materials, are not eluted during GC analysis, the apparent proportions of EPA and DHA are further inflated above the true values, unless properly expressed as mg/g sample. The problems presented by the presence of naturally occurring nontriacylglycerol components or manufacturing additives in encapsulated fish oils require the use of an internal standard. Tricosanoic acid (23:0) has been suggested (21, 22) and tested with both capillary and packed columns. A concerted attack on the problem (23) showed that methyl 23:0 did not coelute with any of the fish oil fatty acid methyl esters when analyzed on a flexible fused silica column coated with a bonded liquid-phase based on the polyglycol, Carbowax-20M (Figure 991.39). The option of calibrating the EPA and DHA peaks against external standard esters must be mentioned, but it should be discouraged on the grounds of the considerable expense of purified EPA and DHA and the known oxidative instability of these 2 compounds once their containers are opened for use (24–26).

The final problem in the analysis of fish oil fatty acids lies in the need to apply corrections to the electronic response of the universally used flame ionization detector to equate peak areas with mass for the wide range of fatty acid chain lengths, C_{14}–C_{24}, present in fish oils. Although these theoretical detector correction factors (4, 27) are relatively unimportant in the analysis of those vegetable oils that are primarily made up of C_{16} and C_{18} fatty acids (28), they are clearly necessary in the analysis of fish oil fatty acids. Because only the theoretical detector factors should be used, the importance of instrument optimization cannot be overemphasized (4). Capsules of Sample 1 for instrument optimization are available from the Charleston Laboratory, National Marine Fisheries Service, PO Box 12607, Charleston, SC 29422.

A particular benefit of the polyglycols as a recommended liquid phase is that this material is homogeneous in chemical composition, and any bleed with use over time does not change the polarity. Experience indicates that the coating thickness may decrease with use, but the loss of fragments of long molecules does not change the polarity of the balance of the coating. The relative retention times (and resolution) of fatty acids of differing type, especially 21:5n-3 and 23:0, therefore, do not change even if the on-column load has to be slightly reduced with time. Overall, the stability of "bonded" polyglycol columns is such that 2 years of useful life can be expected and is often exceeded. A chemically mixed phase may also lose parts of polymer chains without any effect on polarity, but, in the long run, it appears that a portion of one part, the phenyl groups of DB-225 for example, may be affected, redefining the chemical nature of the liquid phase and altering elution patterns. The only caveats for use of the readily available commercial polyglycol columns are that neither 24:0 nor 24:1 coelutes with 22:6n-3 and that 21:5n-3 be resolved from 23:0. The mono-*trans* artifacts of EPA (18) do not interfere with its determination on polyglycols and, indeed, are an accurate measurement of sample abuse. Similar artifacts of DHA have not been investigated as thoroughly.

Some lipid chemists use antioxidants in solvents, but less trouble from contamination generally follows from the liberal use of an inert gas, usually nitrogen, to exclude atmospheric oxygen. Most polyunsaturated fatty acids have induction periods before severe autoxidation begins (24, 25). Nevertheless, some sense of urgency should accompany all analyses of fish oils, especially refined oils that have been removed from the natural matrix where carotenoids, squalene, etc., may be natural antioxidants. The procedure described may take only 2 h to complete, but the increasing use of autosamplers with delayed overnight analysis reinforces the need to exclude oxygen at every step in the procedure.

Of 2964 potential values to be reported in this study, 2346 area percent values and 180 weight values were received. A few collaborators consistently failed to list values for 20:0, 20:2n-6, 20:3n-6, and 20:3n-3; other collaborators consistently listed these components as being present at 0.1–0.4%. It is probable that the first group of collaborators operated their instruments at less than adequate sensitivity. Dixon and/or Grubbs tests identified a total of 109 outlier values among the 2526 values submitted. Area percent outlier values were more common in the reports of Collaborators 15, 16, 18, and 20; each reported data containing outliers in 5 of the 6 analyses. Six collaborators reported no values that were outliers. Of the 12 outlier values for weights of EPA and DHA, 10 were submitted by Collaborator 12, although none of the corresponding area percentages reported by this collaborator were outliers. In initially reporting 2500–10 000 mg of EPA and DHA/g sample, Collaborator 12 obviously made calculation errors. However, because no "correc-

tion" factor that would bring the second set of values more in line with those submitted by others is evident, this collaborator may not have completely redissolved the internal standard after addition of the samples and solvent.

The results of the analysis of Samples 1–6 are summarized in Table 9 and compared with the results of 2 prior collaborative studies that included fish oil as a sample (29). Reproducibility relative standard deviations (RSD_R) are listed in this table as coefficients of variation (CV) for consistency with the previously published data. With few exceptions, the CVs of the current study were equivalent to, or substantially lower than, those of the 2 earlier studies.

In Figure 3, 144 mean area percent values (6 samples, 24 analytes) are plotted against the respective RSD_R values to illustrate the observed relationship between the 2 variables in this study. Almost half of these mean values (67) were for fatty acids present at less than 1% of the total acids, and the RSD_R values are elevated, with a cluster between about 25 and 60. These elevated values may be anticipated for fatty acids such as 22:0, 22:4n-6, 22:5n-6, 24:0, and 24:1, which rarely exceed 0.5% in marine oils. The RSD_R values decline to about 10 for means of fatty acids present at 2% or greater. The highest mean observed (27.5%) had an RSD_R of about 5.

The International Standards Organization has introduced the concepts of repeatability confidence value (r) and reproducibility confidence value (R) (3). As stated in the AOAC guidelines for collaborative study procedures, "...assuming normal distribution, when duplicate measurements are performed, the absolute difference between the results of each of these duplicate measurements is expected to be below r or R in 95% of the cases" (30). Thus, these values represent the 95% confidence limits of the differences between 2 successive analyte concentration estimates. In the analysis of blind duplicate Samples 1 and 6 for area percentages of EPA (r = 0.7) and DHA (r = 0.8), only 1 collaborator reported replicated values of EPA that differed by more than 0.7; 2 collaborators obtained results with differences greater than 0.8 for DHA. These aberrant results are clearly seen in Figure 1.

Table 10 summarizes the precision of the capillary column analysis of the more important fatty acids of fish oils, particularly of the nutritionally important n-3 fatty acids. As noted above, the reproducibility for the major fatty acids equals, or is superior to, that obtained in a prior collaborative study of fish oils using packed GC columns.

Recommendation

We recommend that the capillary column GC method for determination of fatty acids of fish oils and ethyl esters derived from that source, both as area percentages and in absolute weights through the use of 23:0 as internal standard, be adopted first action.

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